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Catalytic Properties of Alcohol Acyltransferase in Different Strawberry Species and Cultivars

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The substrate specificity of alcohol acyltransferase (AAT) enzymes from different strawberry varieties was studied. Proteins with AAT activity from fruits of *Fragaria* × *ananassa* Duch. cv. Oso Grande were purified to apparent homogeneity and used for kinetic studies with different straight-chain alcohols and acyl-CoAs. K_m values obtained for Oso Grande enzyme with six different alcohols, using acetyl-CoA as cosubstrate, decreased with increasing length of the alcohol chain. In similar experiments the increase in the acyl-CoA carbon chain was also found to be correlated with a higher substrate specificity. Heptanol ($K_m = 0.73$ mM) and hexanoyl-CoA ($K_m = 0.41$ mM) were the best substrates for Oso Grande AAT. Comparative catalytic studies were carried out with AAT partially purified extracts from the wild type *Fragaria vesca* and five commercial strawberry varieties: Tudnew, Carisma, Camarosa, Sweet Charlie, and Eris. The specificities of these enzymes toward five selected alcohols and acyl-CoAs reflected interesting cultivar differences.

KEYWORDS: Strawberry fruit; aroma; ester biosynthesis; alcohol acyltransferase; cultivar variation

INTRODUCTION

Fruit aroma is determined by a large number of volatile compounds, the distribution and biosynthesis of which are dependent on many factors, such as cultivar, maturity, and postharvest conditions (*1*, *2*). Strawberry aroma is determined by alcohols, aldehydes, esters, sulfur compounds, and Furaneol derivatives (*3*). Despite exhaustive information regarding strawberry volatile composition, few studies have been done in relation to aroma biosynthesis. Reasons for this limited number of biochemical studies on strawberry are the high content of polyphenols and pectins in unripe and ripe fruits and the low protein content of strawberry fruit, hampering any process of enzyme isolation.

Esters and Furaneol-derived compounds are probably the two most important classes of components in strawberry aroma. Furaneol biosynthesis is currently studied by several research groups. Various sugars have been suggested as precursors for Furaneol synthesis, with fructose being the most likely candidate (*4*−*7*). More recently two aldolase isoenzymes that could be involved in Furaneol biosynthesis have been isolated and characterized in strawberry cv. Elsanta (*8*). Enzymes such as hydrolase, lipoxygenase, hydroperoxide-lyase, and alcohol dehydrogenase involved in the synthesis of lactones, aldehydes, and alcohols have also been described in strawberry (*9*−*11*). These aldehydes and alcohols, besides their direct contribution to fruit aroma, have an important role as precursors in the biosynthesis of esters. Volatile esters resulting from the esterification of an acyl moiety from acyl-CoA onto an alcohol are qualitatively and quantitatively the main components of strawberry aroma. The influence of esters on strawberry aroma makes alcohol acyltransferase (AAT), the enzyme catalyzing the esterification reaction, a key enzyme in strawberry aroma biochemistry. AAT proteins have been studied in several crude fruit extracts (*12*, *13*). Strawberry AAT was partially purified by our group in strawberry cv. Chandler (*14*). The specificity of this enzyme was correlated with the volatile composition of ripe Chandler fruit, suggesting that ester composition is dependent on the properties of the enzyme. In further studies AAT activity was assayed during strawberry ripening and postharvest shelf life, and new data on the enzyme involvement not only in flavor but also in off-flavor generation were provided (*15*). More recently, a novel strawberry alcohol acyltransferase (SAAT) gene has been identified in ripening cv. Elsanta strawberry fruit (*16*). Biochemical evidence for the involvement of the SAAT gene in the formation of fruity esters was provided by characterizing the recombinant protein expressed in *Escherichia coli*.

Despite the knowledge generated in the past few years, we still have a limited understanding of the mechanisms controlling the synthesis of aroma volatiles. Understanding the properties of enzymes involved in the production of aroma volatiles could improve strawberry flavor following shipping and marketing (*1*). In this sense, the aim of this work was to go further in the characterization of kinetic properties and substrate specificity of AAT, comparing the behavior and characteristics of proteins from different strawberry cultivars.

EXPERIMENTAL PROCEDURES

Material. Cv. Oso Grande strawberries (*Fragaria* × *ananassa* Duch) grown in S. Bartolomé de la Torre (Huelva, Spain) were used to obtain

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pure homogeneous AAT protein for the kinetic studies. Five other commercial strawberry varieties with European (Carisma, Tudnew, and Eris) and American origin (Camarosa and Sweet Charlie) and the wild type *Fragaria vesca* were partially purified and used for the substrate specificity studies. Fruits were harvested at commercially ripe stage, selecting for uniformity of size and color development. Strawberry fruits were frozen in liquid N_2 and stored at −20 °C until used.

Chemicals. Tris base, Triton X-100, polyvinyl polypirrolidone (PVPP), polyethylene glycol (PEG), different acyl-CoAs, and all other reagents were supplied by Sigma. Protein standards for SDS-PAGE and size exclusion chromatography were from Amersham Pharmacia Biotech.

Fractionation of Crude Extracts. Crude extract was ultracentrifuged at 250000*g* for 120 min, giving rise to a microsomal pellet. The resulting supernatant was further ultracentrifuged at 350000*g* for 120 min, and a postmicrosomal pellet (PMP) and a soluble cytosolic fraction were obtained. Microsomes were washed with the extraction buffer and resuspended in three different buffers (0.1 M Tris-HCl, pH 8.0; 0.1 M Tris-HCl, 1 M KCl, pH 8.0; and 0.1 M Tris-HCl, 0.1% Triton X-100, pH 8.0), followed by recentrifugation at 250000*g* for 120 min.

AAT Full Purification Procedure. *Step 1.* Strawberry fruits (200 g) and 35 g of PVPP were homogenized with 266 mL of 0.1 M Tris-HCl, 0.1% Triton, and 1 M KCl, pH 8.0. The resulting homogenate was vacuum filtered through Whatman No. 1 filter paper and the residue washed twice with 66 mL of the same buffer. Extract was centrifuged at 27000*g* for 30 min.

Step 2. Triton X-100 was removed from the crude extract by addition of the resin Bio-Beads SM-2 (Bio-Rad) directly into the crude extract (0.2 g of adsorbent/mL of extract) followed by stirring (180 min) and further filtration.

Step 3. $(NH_4)_2SO_4$ was added to the detergent-free extract and the pellet obtained at 20−70% saturation collected and redissolved in 0.1 M Tris-HCl, pH 8.0.

Step 4. The latter enzymatic extract was loaded onto a Sephacryl S-300 (100 × 2.8 cm, Pharmacia Amersham) column and eluted with 0.1 M Tris-HCl, pH 8.0.

Step 5. Active fractions from gel filtration chromatography were pooled and loaded onto a DEAE cellulose DE-52 (6 × 1.5 cm, Whatman) column equilibrated with 0.1 M Tris-HCl, pH 8.0. A stepwise elution was run with the above buffer containing 0, 0.1, and 0.5 M KCl.

Step 6. Active fractions from ion-exchange chromatography were pooled and loaded onto a hydroxyapatite column (1.5 cm × 4.5 cm, CHI II, Bio-Rad) equilibrated with 0.1 M Tris, 10 mM Na_2HPO_4, pH 8.0. The protein fraction with AAT activity was eluted by a linear gradient of 80 mL of 10−150 mM Na_2HPO_4 in 0.1 M Tris-HCl. Active fractions were pooled and concentrated by using a membrane filter of 50 kDa (Ultrafree centrifugal device, Millipore) up to a final volume of 150 μL.

Step 7. The last purification step consisted of an affinity chromatography. A chromatographic column specific for enzymes using hexanol was prepared with ECH−Sepharose 4B (Pharmacia Amersham Biotech.) and amino-1-hexanol in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide. The enzymatic extract obtained in step 6 was conditioned to 10 mM Tris, loaded onto the hexanol−Sepharose affinity column, and eluted by a linear gradient of 30 mL of 0−0.5 M KCl.

AAT Partial Purification Procedure. All procedures were performed at 0−4 °C.

Step 1. Frozen strawberry fruits (30 g) and 5 g of PVPP were homogenized with 40 mL of extraction buffer (100 mM Tris-HCl, pH 8.0, 0.1% Triton X-100, 1 M KCl, 10% PEG-8000). The resulting homogenate was vacuum filtered through Whatman No. 1 filter paper and the residue washed twice with 10 mL of the same buffer. Extracts were centrifuged at 20000*g* for 20 min.

Step 2. PEG-8000 was added to the crude extract, and the pellet obtained at 10−50% saturation was collected.

Step 3. The PEG pellet was redissolved in 50 mM Tris-HCl and loaded onto a DEAE-Sepharose fast flow column (Ammersham Pharmacia, Uppsala, Sweden) equilibrated with the same buffer. A stepwise elution was run with the above buffer containing 0, 0.15, and 0.5 M KCl.

SDS-PAGE. SDS-PAGE was performed with a Hoefer mini-gel apparatus (Pharmacia Biotech) in a discontinuous system. Polypeptides were resolved on 10% (w/v) polyacrylamide 1.0-mm-thick slab gels containing 0.1% sodium docecyl sulfate (separating gel) (ratio of acrylamide to *N*,*N*′-methylenebisacrylamide 37.5:1) according to the method of Laemmli (1970) with 4% (w/v) polyacrylamide for the stacking gel. All samples were preincubated in the presence of SDS sample buffer [250 mM Tris, pH 6.8, 10% SDS, 40% (w/v) glycerol, 20% (w/v) mercaptoethanol, 0.005% (w/v) Bromophenol Blue] for 3 min at 100 °C before being loaded onto the gel. Gels were stained with silver (*17*).

Assay of AAT Activity. The standard assay mixture consisted of 0.85 mL of 0.5 M Tris-HCl, pH 8.0, buffer containing 11.6 mM $MgCl_2$, 0.3 mM acetyl-CoA, 10 mM butanol, and 0.15 mL of the enzyme solution. The mixture was incubated at 35 °C for 15 min, and then 50 μL of 20 mM 5,5′-dithiobis(nitrobenzoic acid) (DTNB) was added. Blank samples containing all of the constituents of the standard assay mixture except the alcohol moiety were carried in parallel with each AAT assay. The increase in absorbance at 412 nm over time due to the yellow thiophenol product formed by the reaction of DTNB with the free CoA-SH liberated was measured.

Protein Determination. Protein was measured according to the method described by Bradford (*18*), using the Sigma Bradford reagent with crystalline BSA as the standard protein.

Specificity Studies. To determine the activity of the partially purified enzymes from Tudnew, Carisma, Camarosa, Sweet Charlie, Vesca, and Eris fruits, a set of experiments was carried out in which AAT activity was assayed according to the described assay conditions but using saturating concentrations of all alcohols and acyl-CoAs tested. Thus, hexanol (15 mM) was used as cosubstrate with acetyl-CoA (7 mM), propionyl-CoA (2 mM), butanoyl-CoA (2 mM), pentanoyl-CoA (2 mM), and hexanoyl-CoA (2 mM). Similarly, acetyl-CoA (7 mM) was used as cosubstrate with ethanol (400 mM), propanol (150 mM), butanol (200 mM), pentanol (40 mM), and hexanol (15 mM).

Kinetic Studies. For the determination of K_m and V_{max} the described standard assay mixture was used. To calculate the AAT kinetic parameters with different acyl-CoAs, hexanol was used as cosubstrate at saturating concentration (15 mM). Concentrations of acyl-CoAs were in the ranges of 0−3.5 mM for acetyl- and propionyl-CoA, 0−3 mM for butanoyl-CoA, and 0−2 mM for pentanoyl- and hexanoyl-CoA. When AAT kinetic parameters with different alcohols were calculated, acetyl-CoA was used as cosubstrate at saturating concentration (7 mM). Concentrations of alcohols were in the ranges of 0−400 mM for ethanol, 0−160 mM for propanol, 0−200 mM for butanol, 0−40 mM for pentanol and hexanol, and 0−1 mM for heptanol. In these kinetic experiments 0.3 μg of homogeneous AAT protein was used in each assay.

RESULTS AND DISCUSSION

Purification of AAT from Cv. Oso Grande Strawberry Fruits. The purification process described under Experimental Procedures differs greatly from that previously used in the preliminary partial purification of strawberry AAT (*14*). Preparation of crude extract has been considerably simplified by using 1 M KCl, 0.1% Triton X-100, and 6% PVPP in the extraction buffer. Low levels of AAT activity found in crude extracts prepared without Triton X-100 suggested that strawberry AAT could be a membrane-bound enzyme. This point was confirmed by results obtained in the subcellular localization studies. AAT activity was assayed in the three subcellular fractions obtained according to procedure described under Experimental Procedures. AAT activity was present only in the microsomal fraction. This activity was not solubilized from the microsomal pellet by increasing the ionic strength with 0.1 M Tris-HCl, 1 M KCl, pH 8.0, and recentrifugation at 250000*g* for 120 min, but was

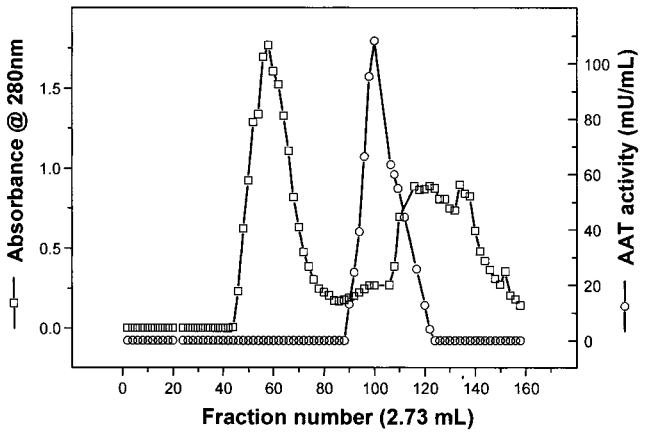

Figure 1. Elution profile of AAT from Oso Grande strawberry fruits on a Sephacryl S-300 (100 × 2.8 cm) column. Flow rate was regulated to 27 mL/h, and fractions of 2.73 mL were collected and assayed for protein and AAT activity.

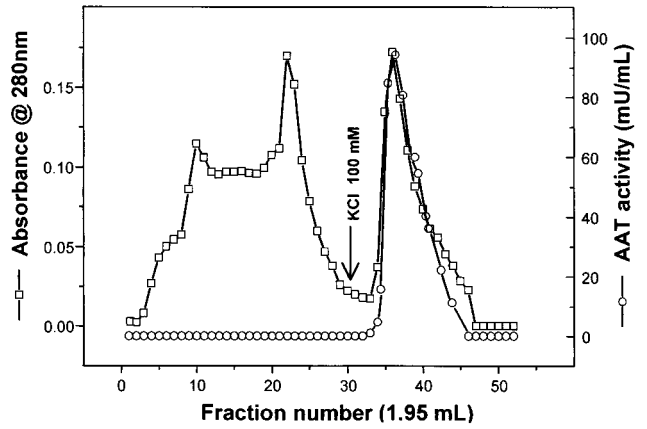

Figure 2. Elution profile of AAT from Oso Grande strawberry fruits on a DEAE anion exchange (6 cm × 2.8 cm) column. Fractions of 1.95 mL were collected at a flow rate of 16.4 mL/h.

efficiently extracted (100%) when resuspension was done in 0.1 M Tris-HCl, 0.1% Triton X-100, pH 8.0. These data demonstrate that strawberry AAT is a membrane-bound enzyme. On the other hand, although addition of 1 M KCl did not increase significantly (8%) AAT extraction, it was added to the extraction buffer to avoid gelification of pectic material in the crude extract that could interfere with the filtration process. The first step in the purification procedure was concentration of crude extract proteins by precipitation with amonium sulfate. Before fractionation with amonium sulfate, Triton X-100 was removed from the crude extract to facilitate protein precipitation by avoiding formation of a detergent floating layer that traps AAT proteins. Removal of Triton X-100 was also important to reduce possible interferences of detergent during further chromatographic procedures. Although a complete elimination of detergent was not achieved, by using Bio-Beads adsorbent up to 80% of the Triton X-100 present in the crude extract was eliminated. Precipitated proteins between 20 and 70% $(NH_4)_2SO_4$ saturation, representing 45% of initial protein content and 43% of total AAT activity in crude extracts, were applied to a Sephacryl S-300 size exclusion chromatography column, where a single peak of active AAT protein was obtained (**Figure 1**). Protein fractions with AAT activity were pooled and loaded onto a DEAE anion-exchange column and eluted at 0.1 M KCl as a single peak of protein (**Figure 2**). Active protein was further purified by using a hydroxyapatite column. As shown in the

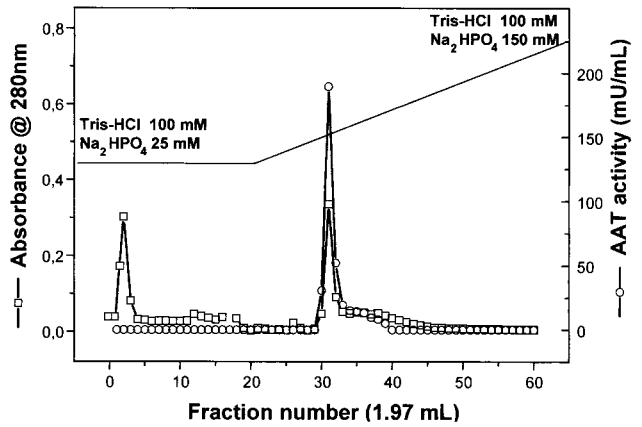

Figure 3. Elution profile of AAT from Oso Grande strawberry fruits on a hydroxyapatite (1.5 cm × 4.5 cm) column. Flow rate was 16.86 mL/h.

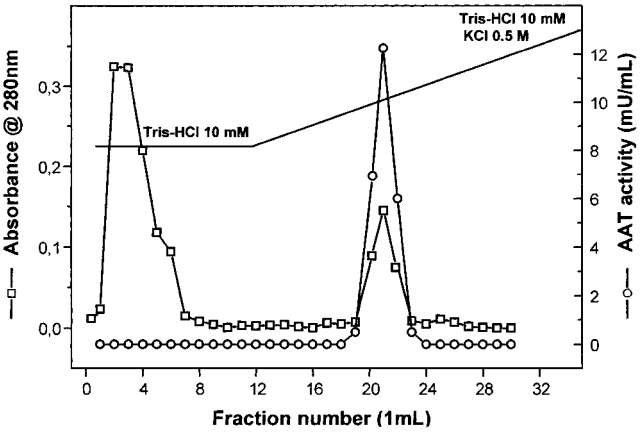

Figure 4. Elution profile of AAT from Oso Grande strawberry fruits on a hexanol–Sepharose 4B (1.5 cm × 4.5 cm) column. Flow rate was 10 mL/h.

typical elution profile (**Figure 3**), AAT activity remained tightly bound to the hydroxyapatite column even after prolonged washing with equilibrium buffer, and it was eluted only by increasing the Na_2HPO_4 concentration in a linear gradient (25−150 mM). As shown in **Figure 3**, enzyme activity coincided with the very sharp second peak of protein, indicating that a practically pure enzyme preparation had been obtained. Nevertheless, this protein eluted from the hydroxyapatite column was found by SDS-PAGE to be contaminated with a small amount of other proteins. To purify the enzyme further, several methods of affinity chromatography were examined, and finally a chromatographic column specifically designed for hexanol-using enzymes was employed to obtain homogeneous pure strawberry AAT protein. A typical elution profile of AAT on hexanol−Sepharose is shown in **Figure 4**. SDS-PAGE of the purified enzyme gave a single band (data not shown) indicating that the enzyme was purified to apparent homogeneity. The final yield of this complex purification procedure was quite low (3.7%) but very similar to that achieved in the purification process of AAT from *Saccharomyces cerevisiae* (*19*). The main reason for this low yield is AAT unstability in the absence of −SH group protectors, a protein characteristic that it was already described in preliminary studies (*14*). Protectors of −SH groups such as mercaptoethanol or DTT were not used throughout the purification process because they would interfere in the described AAT spectrophotometric assay, which is based on the reaction between DTNB and the −SH residue of the corresponding acyl-CoA. With the exception of molecular weight

Table 1. Kinetic Parameters Obtained for Strawberry Cv. Oso Grande Pure AAT Protein with Six Different Alcohols Using Acetyl-CoA as Cosubstrate at Saturating Concentration (7 mM)

alcohol	carbon no.	K_m (mM)	V_{max} (nmol/mL·min)
ethanol	C2:0	45.16	16.78
propanol	C3:0	57.02	108.2
butanol	C4:0	25.09	43.29
pentanol	C5:0	20.17	58.41
hexanol	C6:0	1.93	105.6
heptanol	C7:0	0.73	132.98

Table 2. Kinetic Parameters Obtained for Strawberry Cv. Oso Grande Pure AAT Protein with Five Different Acyl-CoAs Using Hexanol as Cosubstrate at Saturating Concentration (15 mM)

acyl-CoA	carbon no.	K_m (mM)	V_{max} (nmol/mL·min)
acetyl-CoA	C2:0	2.60	267.38
propanoyl-CoA	C3:0	3.09	174.13
butanoyl-CoA	C4:0	0.69	147.27
pentanoyl-CoA	C5:0	0.76	216.91
hexanoyl-CoA	C6:0	0.41	134.04

(M_r), the catalytic and physicochemical properties of this purified strawberry AAT are in good agreement with those previously described for the AAT enzyme partially purified from cv. Chandler strawberries. In the present study M_r calculated by SDS-PAGE was 48 kDa, quite similar to the 54 kDa estimated for the SAAT recombinant enzyme described recently by Aharoni et al. (*16*). Differences with the M_r previously calculated by gel filtration using an FPLC system (*14*) can be explained by formation of detergent−protein complexes with Triton X-100. This phenomenon was first described for AAT from *S. cerevisiae*, which showed an apparent M_r of 270 kDa by gel filtration chromatography, whereas the value obtained by SDS-PAGE was 60 kDa (*19*).

Kinetic Studies. The main objective of this study was the characterization of kinetic properties and substrate specificity of strawberry AAT. K_m and V_{max} values were determined from the Michaelis−Menten equation by a Lineweaver−Burk plot. **Table 1** shows K_m and V_{max} values found for six straight-chain alcohols using acetyl-CoA as cosubstrate at saturating concentration. As a general rule K_m values decreased with increasing length of the alcohol carbon chain. The enzyme showed the highest K_m (57.02 mM) with propanol, an exception to this rule, whereas the lowest K_m value corresponded to heptanol (0.73 mM). Data obtained are in agreement with the parallel increase of activity found by Aharoni et al. (*16*) when SAAT recombinant enzyme was incubated with different alcohols. This property is also similar to those of AAT from *S. cerevisiae* (*19*) and the brewers lager yeast enzyme (*20*). On the contrary, published data in relation to banana AAT specificity (*21*) indicated a higher AAT activity toward butanol, amyl, and isoamyl alcohols than toward hexanol. Although several studies have reported a correlation between AAT specificity and volatile composition of different fruit varieties and fermented products (*21*, *22*), the wide range of alcohols and acyl-CoAs accepted by strawberry AAT cannot completely explain the ester composition of strawberry aroma. In this sense, propanol and heptanol with very different K_m values are both very poorly represented as acetate esters in the aroma of most strawberry varieties. In fact, although the enzyme exhibited the lowest K_m value with heptanol using acetyl-CoA as acyl donor, the corresponding ester, heptyl acetate, has never been described as a typical component of strawberry aroma. As was pointed out in previous studies (*14*, *23*, *24*), two main factors could be involved in determining volatile ester composition in fruits: the inherent properties of the AAT enzyme and the availability of substrates. Thus, it is important to point out that two of the most relevant biochemical pathways involved in the biosynthesis of acyl-CoAs and alcohols in fruits, β-oxidation and the lipoxygenase pathway, preferentially generate products with an even number of carbons, which could explain the lower percentage of propyl and heptyl esters found in comparison with the higher amount of butyl and hexyl esters. In a similar set of experiments the K_m and V_{max} values of AAT with five different acyl-CoAs using hexanol as cosubstrate were calculated (**Table 2**). In most cases, these K_m values listed in **Table 2** are significantly lower than those previously calculated with the alcohols (**Table 1**). This higher specificity of the enzyme for the acyl moiety seems to indicate that the alcohol is the rate-limiting factor in the formation of most esters in strawberries. A similar conclusion was raised by Minetoki et al. (*19*) after their study of the kinetic properties of AAT from *S. cerevisiae*. Results obtained here are also consistent with those reported for banana by Wyllie and Fellman (*24*). As was described for the alcohols, the increase in the acyl-CoA carbon chain is also correlated with a higher substrate specificity, hexanoyl-CoA (K_m = 0.41 mM) being the best substrate for strawberry AAT. The K_m value of AAT calculated for acetyl-CoA (2.6 mM) is considerably higher than that reported by Aharoni et al. (*16*) for SAAT recombinant enzyme (104 μM) and by Harada et al. (*12*) for banana AAT (50 μM). The K_m obtained in this study also differs from that calculated for the partially purified enzyme from cv. Chandler strawberry (65 μM). These differences could be attributed to the different cosubstrates employed in each of those studies (hexanol, octanol, isoamyl alcohol, and butanol).

Substrate Specificity. To compare the substrate specificity of AAT proteins from different strawberry varieties, a simplified purification protocol was designed. This partial purification procedure consisted of a protein fractionation with PEG-8000 and further anion-exchange chromatography onto a fast flow DEAE-Sepharose column. The AAT specific activity of the extracts obtained was ∼500−600 mU/mL with a purification factor of ∼10−12-fold. Cv. Oso Grande strawberry specificity was studied with this procedure. A set of experiments were carried out in which the activity of the partially purified enzyme with several alcohols, at the saturating concentration previously determined by Michaelis−Menten curves, was assayed. Results showed a wide range of AAT relative activities: ethanol (18%), propanol (30%), butanol (38%), pentanol (45%), hexanol (86%), and heptanol (100%). These data are in good agreement with those previously obtained in the kinetic studies carried out with homogeneous AAT protein. Thus, the lowest K_m value calculated for the homogeneous AAT protein with heptanol correlates with the highest relative activity found for the partially purified enzyme with the same alcohol. This correlation between K_m and relative AAT activity was also valid for hexanol, pentanol, and butanol. The higher than expected relative activity found for propanol, with a slightly higher K_m than that of ethanol, could be explained by the higher V_{max} determined for this alcohol (108.2 nmol/mL·min) compared to that found for ethanol (16.7 nmol/mL·min). From these results it can be concluded that the described assay of relative activity with partially purified enzyme is a valid method to study and compare the substrate specificities of AAT proteins from different sources.

AAT proteins from five commercial strawberry varieties, one from California (Camarosa), one from Florida (Sweet Charlie), one from Italy (Eris), two from Spain (Tudnew and Carisma), and the wild type (*Fragaria vesca*), were partially purified and

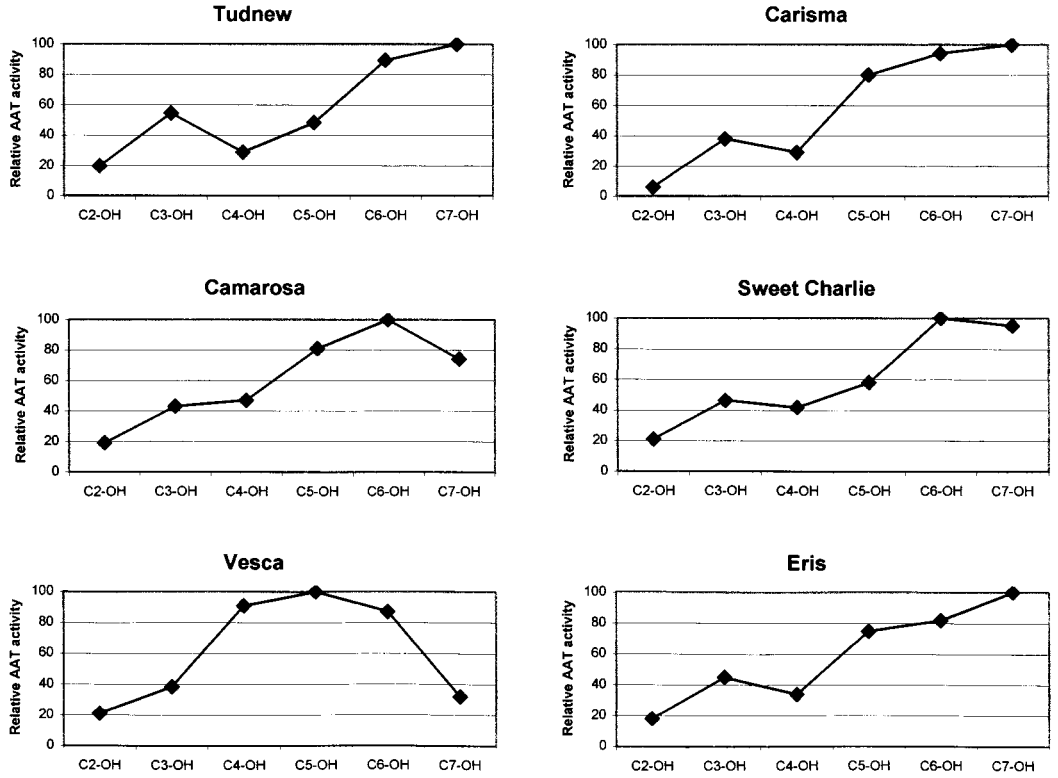

Figure 5. Relative AAT activity toward straight-chain alcohols of partially purified extracts from six strawberry varieties.

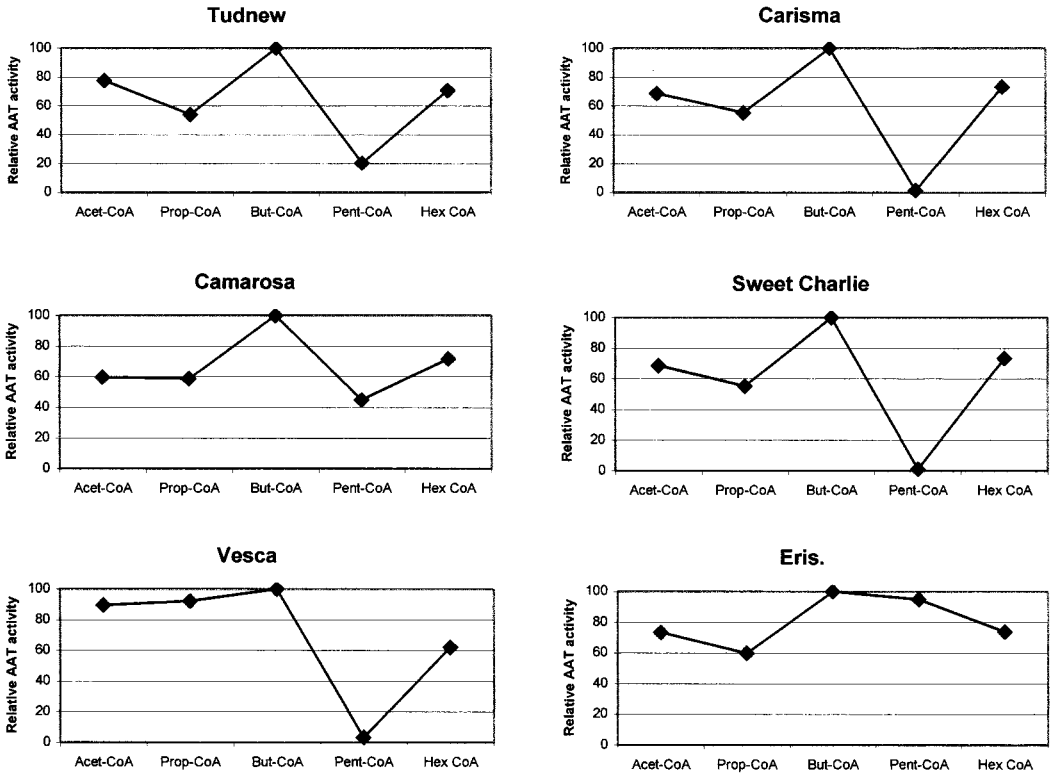

Figure 6. Relative AAT activity toward five straight-chain acyl-CoAs of partially purified extracts from six strawberry varieties.

their substrate specificities tested. Results obtained are shown in **Figures 5** and **6**. Most commercial varieties showed a very similar pattern of activity toward straight-chain alcohols, with increasing AAT relative activity from C2:0 to C7:0 and a relatively lower AAT activity using butanol. Heptanol was the best substrate for the three European varieties, whereas hexanol was the preferred alcohol for the American cultivars. The wild type, *F. vesca*, showed a completely different pattern in which 100% of AAT activity was obtained with pentanol, and very high activity levels were also found with butanol and hexanol as substrates. The specificity of the enzyme toward five selected acyl-CoAs also reflected interesting varietal differences. Compared to the commercial varieties, the wild type *F. vesca* showed unusually high relative activity values with short-chain acyl-

CoAs such as acetyl- and propionyl-CoA. Pentanoyl-CoA was, with the exception of the Eris variety, the worst substrate for all tested varieties.

Although some compounds such as methyl and ethyl butanoate and hexanoate quantitatively comprise the bulk of the volatile esters produced by most fresh commercial strawberries, it is clear that there are significant differences in the specific ester distribution of each strawberry cultivar (*1*, *25*). These differences in the volatile ester profile could be related to the different specificities of the corresponding AAT proteins. In this sense, recent studies have demonstrated that cultivar variation in two aroma key enzymes, such as alcohol dehydrogenase and pyruvate decarboxylase, could explain the different susceptibilities of strawberry varieties to postharvest disorders such as off-flavor development during controlled atmosphere/modified atmosphere storage (*26*, *27*). In our opinion, in the same way that differences in the AAT activity profile during fruit development and ripening have been correlated with the different acceptabilities of strawberry varieties by the consumer (*15*), a deeper knowledge of the properties of AAT proteins from different strawberry cultivars may lead to genetic or environmental manipulations to preserve and enhance strawberry aroma during postharvest handling.

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